IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
ASTATKE *et al.*
Appl. No.: 09/608,066
Filed: June 30, 2000

Art Unit: 1655
Examiner: Taylor, J.
Atty. Docket: 0942.4990001/RWE/BJD

#12 Amdt. A 8/18/01

For: **Compositions and Methods for Enhanced Sensitivity and Specificity of Nucleic Acid Synthesis**

Amendment and Reply Under 37 C.F.R. § 1.111

Commissioner for Patents
Washington, DC 20231

Sir:

In reply to the non-final Office Action dated February 14, 2001 (Paper No. 16), Applicants submit the following remarks. This Amendment and Reply is provided in the following format:

(A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;

(B) Starting on a separate page, appropriate remarks and arguments. *See* 37 C.F.R. § 1.121 and MPEP § 714; and

(C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a),

and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Claims:

Please amend the claims as follows:

Please cancel claims 1-11, 30-31 and 35-59, without prejudice to or disclaimer of the subject matter contained therein. Applicants reserve the right to prosecute these claims in one or more continuing applications.

Please substitute the following claim 12 for currently pending claim 12:

12. (Once amended) A method for synthesizing a nucleic acid molecule comprising:

mixing at least one enzyme with polymerase activity with one or more nucleic acid inhibitors and one or more templates, wherein said one or more inhibitors each comprises a 5' portion and a 3' portion, said 3' portion comprising one or more deoxyribonucleotides or derivatives thereof and said 5' portion comprising one or more ribonucleotides or derivatives thereof; and

incubating said mixture under conditions sufficient to synthesize one or more first nucleic acid molecules complementary to all or a portion of said templates.

Please substitute the following claim 19 for currently pending claim 19:

19. (Once amended) A method for amplifying a nucleic acid molecule comprising:

mixing at least one nucleic acid inhibitor with one or more enzymes with polymerase activity and one or more templates, wherein said inhibitor comprises a 5' portion and a 3' portion, said 3' portion comprising one or more deoxyribonucleotides or derivatives thereof and said 5' portion comprising one or more ribonucleotides or derivatives thereof; and

incubating said mixture under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said templates.

Please substitute the following claim 25 for currently pending claim 25:

25. (Once amended) A method for sequencing a nucleic acid molecule, comprising:

mixing at least one nucleic acid molecule to be sequenced with one or more nucleic acid inhibitors, one or more enzymes having polymerase activity, and one or more terminating agents, wherein said one or more inhibitors each comprises a 5' portion and a 3' portion, said 3' portion comprising one or more deoxyribonucleotides or derivatives thereof and said 5' portion comprising one or more ribonucleotides or derivatives thereof;

incubating said mixture under conditions sufficient to synthesize a population of molecules complementary to all or a portion of said molecules to be sequenced; and

separating said population to determine the nucleotide sequence of all or a portion of said molecule to be sequenced.

Please substitute the following claim 32 for currently pending claim 32:

32. (Once amended) A method for amplifying a double stranded DNA molecule, comprising:

(a) providing a first and second primer, wherein said first primer is complementary to a sequence within or at or near the 3'-termini of the first strand of said DNA molecule and said second primer is complementary to a sequence within or at or near

the 3'-termini of the second strand of said DNA molecule and one or more nucleic acid inhibitors, wherein said one or more inhibitors each comprises a 5' portion and a 3' portion, said 3' portion comprising one or more deoxyribonucleotides or derivatives thereof and said 5' portion comprising one or more ribonucleotides or derivatives thereof, under conditions such that said inhibitors prevent or inhibit nucleic acid synthesis;

(b) hybridizing said first primer to said first strand and said second primer to said second strand to form hybridized molecules;

(c) incubating said hybridized molecules under conditions sufficient to allow synthesis of a third DNA molecule complementary to all or a portion of said first strand and a fourth DNA molecule complementary to all or a portion of said second strand;

(d) denaturing said first and third strand, and said second and fourth strands; and

(e) repeating (a) to (c) or (d) one or more times.

Please substitute the following claim 33 for currently pending claim 33:

33. (Once amended) A method of preparing cDNA from mRNA, comprising

mixing one or more mRNA templates, one or more reverse transcriptases, and with one or more nucleic acid inhibitors, wherein said one or more inhibitors each comprises a 5' portion and a 3' portion, said 3' portion comprising one or more deoxyribonucleotides or derivatives thereof and said 5' portion comprising one or more ribonucleotides or derivatives thereof; and

incubating said mixture under conditions sufficient to synthesize one or more cDNA molecules complementary to all or a portion of said templates.

Please enter the following new claims 60-88

60. (New) The method of any one of claims 12, 19, 25, 32 and 33, wherein all or a portion of said 3' portion of said inhibitor is capable of base pairing to all or a portion of said 5' portion of said inhibitor.

61. (New) The method of any one of claims 12, 19, 25, 32 and 33, wherein said 5' portion of said inhibitor forms a 5' overhang.

62. (New) The method of any one of claims 12, 19, 25, 32 and 33, wherein said nucleic acid inhibitor is in the form of a hairpin comprising at least a stem structure.

63. (New) The method of claim 62, wherein said stem structure comprises a series of contiguous ribonucleotides that are basepaired with or hybridized to a series of contiguous deoxyribonucleotides.

64. (New) A method for synthesizing a nucleic acid molecule comprising:

mixing at least one enzyme with polymerase activity with one or more double stranded nucleic acid inhibitors and one or more templates; and

incubating said mixture under conditions sufficient to synthesize one or more first nucleic acid molecules complementary to all or a portion of said templates.

65. (New) The method of claim 64, wherein said mixing is accomplished under conditions to prevent nucleic acid synthesis and/or to allow binding of said double stranded nucleic acid inhibitor to said enzyme with polymerase activity.

66. (New) The method of claim 64, wherein said synthesis of said first nucleic acid molecule is accomplished under conditions sufficient to reduce the inhibitory affect of said double stranded nucleic acid inhibitor, and/or to inhibit, reduce, substantially reduce, or eliminate binding of said double stranded nucleic acid inhibitor to said enzyme with polymerase activity.

67. (New) The method of claim 64, wherein said synthesis is accomplished in the presence of at least one component selected from the group consisting of one or more nucleotides and one or more primers.

68. (New) The method of claim 64, wherein said template is a double stranded nucleic acid molecule.

69. (New) The method of claim 64, further comprising incubating said one or more first nucleic acid molecules under conditions sufficient to make one or more second nucleic acid molecules complementary to all or a portion of said first nucleic acid molecules.

70. (New) A method for amplifying a nucleic acid molecule comprising:

mixing at least one double stranded nucleic acid inhibitor with one or more enzymes with polymerase activity and one or more templates; and

incubating said mixture under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said templates.

71. (New) The method of claim 70, wherein said mixing is accomplished under conditions sufficient to prevent nucleic acid amplification and/or to allow binding of said double stranded nucleic acid inhibitor to said enzyme with polymerase activity.

72. (New) The method of claim 70, wherein said amplifying is accomplished under conditions sufficient to denature said double stranded nucleic acid inhibitor or reduce the ability of the inhibitor to inhibit amplification.

73. (New) The method of claim 70, wherein said amplifying is accomplished in the presence of at least one component selected from the group consisting of one or more nucleotides and one or more primers.

74. (New) The method of claim 70, wherein said template is a double stranded nucleic acid molecule.

75. (New) A method for sequencing a nucleic acid molecule, comprising:

mixing at least one nucleic acid molecule to be sequenced with one or more double stranded nucleic acid inhibitors, one or more enzymes having polymerase activity, and one or more terminating agents;

incubating said mixture under conditions sufficient to synthesize a population of molecules complementary to all or a portion of said molecules to be sequenced; and

separating said population to determine the nucleotide sequence of all or a portion of said molecule to be sequenced.

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76. (New) The method of claim 75, wherein said mixing is accomplished under conditions sufficient to prevent synthesis and/or to allow binding of said double stranded nucleic acid inhibitor to said enzyme with polymerase activity.

77. (New) The method of claim 75, wherein said synthesis is accomplished under conditions sufficient to denature said double stranded nucleic acid inhibitor and/or to reduce the inhibitory affect of said double stranded nucleic acid inhibitor.

78. (New) The method of claim 75, wherein said synthesis is accomplished in the presence of at least one component selected from the group consisting of one or more nucleotides and one or more primers.

79. (New) The method of claim 75, wherein said molecule to be sequenced is a double stranded nucleic acid molecule.

80. (New) A method for amplifying a double stranded DNA molecule, comprising:

(a) providing a first and second primer, wherein said first primer is complementary to a sequence within or at or near the 3'-termini of the first strand of said DNA molecule and said second primer is complementary to a sequence within or at or near the 3'-termini of the second strand of said DNA molecule and one or more double stranded nucleic acid inhibitors, under conditions such that said double strandede inhibitors prevent or inhibit nucleic acid synthesis;

(b) hybridizing said first primer to said first strand and said second primer to said second strand to form hybridized molecules;

(c) incubating said hybridized molecules under conditions sufficient to allow synthesis of a third DNA molecule complementary to all or a portion of said first strand and a fourth DNA molecule complementary to all or a portion of said second strand;

(d) denaturing said first and third strand, and said second and fourth strands; and

(e) repeating (a) to (c) or (d) one or more times.

81. (New) A method of preparing cDNA from mRNA, comprising

mixing one or more mRNA templates with one or more reverse transcriptases, and with one or more double stranded nucleic acid inhibitors; and

incubating said mixture under conditions sufficient to synthesize one or more cDNA molecules complementary to all or a portion of said templates.

82. (New) The method of claim 81, wherein said mixing is accomplished under conditions sufficient to prevent nucleic acid synthesis and/or allow binding of said one or more double stranded nucleic acid inhibitors to said reverse transcriptase.

83. (New) A method for synthesizing a nucleic acid molecule comprising:

mixing at least one enzyme with reverse transcriptase activity with one or more double stranded nucleic acid inhibitors and one or more templates; and

incubating said mixture under conditions sufficient to synthesize one or more first nucleic acid molecules complementary to all or a portion of said templates.

84. (New) The method of claim 83, wherein said mixing is accomplished under conditions to prevent nucleic acid synthesis and/or to allow binding of said double stranded nucleic acid inhibitor to said enzyme with reverse transcriptase activity.

85. (New) The method of claim 83, wherein said synthesis of said first nucleic acid molecule is accomplished under conditions sufficient to reduce the inhibitory affect of said double stranded nucleic acid inhibitor, and/or to inhibit, reduce, substantially reduce, or eliminate binding of said double stranded nucleic acid inhibitor to said enzyme with reverse transcriptase activity.

86. (New) The method of claim 83, wherein said synthesis is accomplished in the presence of at least one component selected from the group consisting of one or more nucleotides and one or more primers.

87. (New) The method of claim 83, wherein said template is a double stranded nucleic acid molecule.

88. (New) The method of claim 83, further comprising incubating said one or more first nucleic acid molecules under conditions sufficient to make one or more second nucleic acid molecules complementary to all or a portion of said first nucleic acid molecules.

Remarks

I. Support for Amendments

Support for the foregoing amendments to the claims may be found throughout the specification as originally filed, either inherently or explicitly. Specifically, support for the amendments to claims 12, 19, 25, 32 and 33, as well as for new claims 60-63, may be found in the specification at page 6, lines 14-26; at page 7, lines 3-12; at page 8, lines 13-20; at page 21; at pages 26-27; and throughout the Examples at pages 40-56. Support for new claims 64-69 may be found at pages 7-8, at page 26, line 13, in Example 7 at pages 44-51, and in claims 12-17 as originally filed; support for new claims 70-79 may be found at pages 7-8, at page 26, line 13, in Example 7 at pages 44-51, and in claims 19-29 as originally filed; support for new claims 80-82 may be found at pages 7-8, at page 26, line 13, in Example 7 at pages 44-51, and in claims 32-34 as originally filed; and support for new claims 83-88 may be found at page 6, lines 26-28, pages 7-8, at page 13, lines 24-26, at page 26, line 13, in Example 7 at pages 44-51, and in claims 12-17 as originally filed. Hence, the foregoing amendments to the claims do not add new matter, and their entry into the present application is respectfully requested.

II. Status of the Claims

By the foregoing amendments, claims 1-11, 30-31 and 35-59 have been cancelled, claims 60-88 are sought to be entered, and claims 12, 19, 25, 32 and 33 have been amended. These amendments do not add new matter. Upon entry of the foregoing amendments, claims 12-29, 32-34 and 60-88 are pending in the application, with claims 12, 19, 25, 32, 33, 64, 70, 75, 80, 81 and 83 being the independent claims.

III. Summary of the Office Action

In the Office Action dated February 14, 2001, the Examiner has made three rejections of the claims. Applicants respectfully offer the following remarks to overcome or traverse each element of this rejection in the Office Action.

IV. The Rejection Under 35 U.S.C. § 102(e) Over Gold Is Traversed

In the Office Action at pages 2-4 , sections 2-3, the Examiner has rejected claims 12-24 under 35 U.S.C. § 102(e) as being anticipated by Gold *et al.*, U.S. Patent No. 6,020,130 (Doc. "B" cited on the Form PTO-892 attached to Paper No. 16; hereinafter "Gold"). Applicants respectfully traverse this rejection as it may apply to the claims that were previously pending. In addition, Applicants respectfully assert that new claims 60-88 which are sought to be added by the foregoing amendments are patentably distinct from the disclosure of Gold; therefore, this rejection should not be applied to these newly added claims.

By the foregoing amendments, claim 12 (and thus the remaining claims depending therefrom) has been amended to recite the use of nucleic acid inhibitors having a 3' portion

with one or more deoxyribonucleotides (dNTs) or derivatives and a 5' portion with one or more ribonucleotides (rNTs) or derivatives. In contrast, the nucleic acid inhibitors disclosed in Gold are composed of dNTs (*see* Gold in the abstract; in the paragraph bridging cols. 5-6; in the description of the figures at cols. 7-8; in the detailed description at cols. 14-15; and in the Examples at cols. 22-31; each of these locations referring to "DNA ligands"). In particular, the definition of "nucleic acid ligand" in Gold at col. 11 does not mention a hybrid dNT-rNT inhibitor nucleic acid, and the definition of "nucleic acid" in Gold at col. 12 indicates that the inhibitors disclosed therein are *either* DNA or RNA (hence, hybrid dNT-rNT molecules are not included within the definition in Gold). Thus, Gold does not expressly or inherently disclose at least one feature of the nucleic acid inhibitors of the presently claimed invention.

Under 35 U.S.C. § 102, a claim can only be anticipated if every element in the claim is expressly or inherently disclosed in a single prior art reference. *See Kalman v. Kimberly Clark Corp.*, 713 F.2d 760, 771 (Fed. Cir. 1983), *cert. denied*, 465 U.S. 1026 (1984). Since Gold does not expressly or inherently disclose nucleic acid inhibitors having a 3' portion with one or more dNTs or derivatives and a 5' portion with one or more rNTs or derivatives, this reference cannot and does not anticipate the claims as amended. Reconsideration and withdrawal of the rejection of claims 12-24 under 35 U.S.C. § 102(e) over Gold therefore are respectfully requested.

V. The Rejection Under 35 U.S.C. § 103(a) Over Gold Is Traversed

In the Office Action at pages 4-6, sections 4-5, the Examiner has rejected claims 32-34 under 35 U.S.C. § 103(a) as being unpatentable over Gold. Applicants respectfully traverse

this rejection as it may apply to the claims that were previously pending. In addition, Applicants respectfully assert that new claims 60-88 which are sought to be added by the foregoing amendments are patentably distinct from the disclosure of Gold; therefore, this rejection should not be applied to these newly added claims.

In proceedings before the Patent and Trademark Office, the examiner bears the burden of establishing a *prima facie* case of obviousness based upon the prior art. *See In re Piasecki*, 223 USPQ 785, 787-88 (Fed. Cir. 1984). The Examiner can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the reference and the knowledge in the art in such a way as to produce the invention as claimed. *See In re Fine*, 5 USPQ2d 1596,1598 (Fed. Cir. 1988). In the present case, this burden has not been satisfied.

Applicants reiterate and incorporate herein the remarks made above concerning the disclosure of Gold. Gold does not expressly or inherently disclose nucleic acid inhibitors having a 3' portion with one or more dNTs or derivatives and a 5' portion with one or more rNTs or derivatives. Thus, Gold is seriously deficient as a primary reference upon which to base a *prima facie* case of obviousness.

The Examiner has pointed to no additional objective evidence or sound scientific reasoning that would cure these deficiencies of Gold. Moreover, the knowledge attributed to one of ordinary skill in the art in the present rejection (*see* Office Action at page 6, second paragraph) is insufficient to overcome the deficiencies of Gold, since "[r]arely . . . will the skill in the art component operate to supply missing knowledge or prior art to reach an obviousness judgment . . . Skill in the art does not act as a bridge over gaps in substantive

presentation of an obviousness case . . . . " *Al-Site Corpn. v. VSI International, Inc.*, 174 F.3d 1308, 1324 (Fed. Cir. 1999).

Hence, Gold does not disclose or suggest the invention as presently claimed, and the information missing from Gold has not been shown to have been readily available in the art at the time of filing of the present application. The skilled artisan therefore would not have been motivated to modify the disclosure of Gold in order to make and use the claimed invention. Thus, the burden required to sustain a *prima facie* case of obviousness has not been met.

In view of the foregoing remarks, Applicants respectfully assert that claims 32-34 are not rendered obvious by the disclosure of Gold. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) are therefore respectfully requested.

VI. The Rejection Under 35 U.S.C. § 103(a) Over Gold In View of Langmore Is Traversed

In the Office Action at pages 6-8, section 6, the Examiner has rejected claims 25-29 under 35 U.S.C. § 103(a) as being unpatentable over Gold in view of Langmore *et al.*, U.S. Patent No. 6,117,634 (Doc. "A" on the Form PTO-892 attached to Paper No. 16; hereinafter "Langmore"). Applicants respectfully traverse this rejection as it may apply to the claims that were previously pending. In addition, Applicants respectfully assert that new claims 60-88 which are sought to be added by the foregoing amendments are patentably distinct from the disclosures of Gold and Langmore, separately or in combination; therefore, this rejection should not be applied to these newly added claims.

Applicants reiterate and incorporate herein the remarks made above concerning the disclosure of Gold and the deficiencies therein. Gold is seriously deficient as a primary reference upon which to base a *prima facie* case of obviousness. These deficiencies are not cured by the disclosure of Langmore, which does not disclose, suggest or otherwise contemplate nucleic acid inhibitors having the structural features recited in the present claims.

Hence, Gold and Langmore, alone or in combination, do not disclose or suggest the invention as presently claimed. The skilled artisan therefore would not have been motivated to combine the disclosures of these references in order to make and use the claimed invention. Absent such suggestion and motivation, the cited references may not be properly combined to render the claimed invention obvious. *See In re Fine*, 5 USPQ2d 1596,1598 (Fed. Cir. 1988). Thus, the burden required to sustain a *prima facie* case of obviousness has not been met.

In view of the foregoing remarks, Applicants respectfully assert that claims 25-29 are not rendered obvious by the disclosures of Gold and Langmore, alone or in combination. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) are therefore respectfully requested.

VII. Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider and withdraw all of the outstanding rejections.

It is believed that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner

believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt entry and favorable consideration of the foregoing amendments and remarks, and allowance of all pending claims, are earnestly solicited.

Respectfully submitted,

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